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(71) Applicant *(for all designated States except US)*: **CROPDESIGN N.V.** [BE/BE]; Technologiepark 3, B-9052 Zwijnaarde (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOUNAGA, Sakina** [FR/BE]; Onafhankelijkheidslaan 30, B-9000 Gent (BE). **INZE, Dirk** [BE/BE]; Driesstraat 18, B-9390 Moorsel-Aalst (BE). **DE VEYLDER, Lieven** [BE/BE]; Popperodedries 42, B-9300 Aalst (BE). **MIRONOV, Vladimir** [RU/BE]; Gentbruggestraat 142, B-9040 Gent (BE).

(74) Agent: **DE CLERCQ, Ann**; De Clercq, Brants & Partners, E. Gevaertdreef 10a, B-9830 Sint-Martens-Latem (BE).

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(54) Title: METHOD FOR IDENTIFYING COMPOUNDS WITH HERBICIDAL OR GROWTH REGULATOR ACTIVITY ON PLANTS

(57) Abstract: The present invention relates to a method for screening and identification of compounds or compositions useful as herbicides, growth regulators or fungicides involving at least the following steps of addition of the compound or composition to be screened or identified to a culture or culture area of a yeast strain transformed with and expressing one or more plant cell cycle control genes or mutants thereof (phytoyeast) as well as to a control yeast strain; and, determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast. The invention further relates to a bilogical screening assay (high throughput system) comprising the use of said phytoyeast expressing plant cell cycle control proteins to identify novel compounds or compositions that affect control yeast or phytoyeast phenotype. The present invention also relates to methods for producing a pesticide, herbicide, plant growth regulator or fungicide, comprising the steps of (a) identifying a compound or composition as defined above or a derivative or homologue thereof, and, (b) mixing said compound, composition, derivative or homologue thereof with an acceptable carrier. The invention also relates to the use of said compounds or compositions for inhibiting or stimulating plant growth and/or for increasing crop yield and/or for preventive or curative protection of the plant against fungal infection.

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METHOD FOR IDENTIFYING COMPOUNDS WITH HERBICIDAL OR GROWTH REGULATOR ACTIVITY ON PLANTS

The present invention relates to screening methods for the identification of compounds and compositions useful as new herbicides or growth regulators. More specifically, the invention relates to screening assays using transformed yeast cells as host that express plant cell cycle control genes to identify novel agrochemicals that abolish, retard or stimulate growth. These cells can be used to develop a high throughput assay system useful in the large scale screening of natural or synthetic libraries of compounds for specific biological activities.

In recent years, the rate of success of new herbicides has slowed down and little new herbicides have been discovered. The search for new biologically active compounds using new technologies such as functional genomics, combinatorial chemistry and bioinformatics will have a large impact on the discovery of new herbicides. As a result of developments in the field of combinatorial chemistry, large libraries containing hundreds of thousands of compounds are produced. With the availability of such libraries has come a need for the development of relatively rapid and precise methods for large-scale screening.

Advances in molecular biology have provided the means to transform organisms to contain and express foreign genes. Such genes can be transformed into the organism (yeast) to affect its function.

The orderly progression of the eukaryotic cell cycle is precisely regulated by a number of cell division cycle (cdc) control proteins. In animals and plants several cyclin-dependent kinases (CDKs) have been found to mediate the progression through the cell cycle. The activation of these kinases requires at least association with a regulatory cyclin subunit. The activities of the CDKs are controlled by several mechanisms such as transcription of CDK genes, association of CDKs with specific cyclins, as well as both positive or negative acting phosphorylation or dephosphorylation and cell cycle dependent proteolysis of cell cycle control proteins. Other regulatory proteins involved in CDK activity are Suc1/CKS which are proteins that associate with the CDKs and probably act as docking factor for both positive and negative regulators of kinase activity and the cyclin-dependent kinase inhibitors (CKIs).

In fission and budding yeast (*Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively) there is only one multifunctional cyclin-dependent kinase ($p34^{CDC2}$ and $p34^{CDC28}$ respectively, also denoted Cdc2 and CDC28). This is a serine-threonine protein kinase (hereafter called Cdc2 to denote the protein form either yeast) which controls cell cycle progression mainly at two transition points, late G1 before DNA synthesis and G2/M transition. The same Cdc2 kinase is responsible for both the transition into S phase and M phase through association with different cyclins. The level of expression of Cdc2 is generally constitutive throughout most eukaryotic cell cycles, with the relative activity of the enzyme being controlled during the cell cycle through a number of positive and negative signals. The activity of Cdc2 is directly regulated by Cdk-activating kinase (CAK) (activates Cdc2 by phosphorylation of Thr161), wee1 (inhibits Cdc2 by phosphorylation of Thr14 and Tyr15), cdc25 (activate Cdc2 through dephosphorylation of Thr14 and Tyr15), Suc1/Cks1 (is thought to function as docking factor on the CDK proteins for both positive and negative regulators of kinase activity), CKIs, and cyclin proteolysis.

Cell cycle mutations in fission yeast can affect the phenotype of the cell. For example mutations which cause cells to enter mitosis prematurely (cells divide when they reach a length only half that of normal cells) give a "wee" phenotype giving rise to yeast cells having a reduced cell size compared to wild type, whereas mutations that cause the arrest of the cell division, cause cell elongation when compared to wild type and give rise to "Cdc" phenotype.

Conditional-lethal *cdc2* mutants have been identified in both *S. pombe* and *S. cerevisiae*. Such mutants fail to survive or fail to grow at non-permissive growth conditions. For example; the conditional-lethal mutant may be temperature sensitive, i.e. the mutant will function normally at permissive temperatures, but fail to function at restrictive temperatures.

In the plant species *Arabidopsis thaliana*, there are two well-characterised CDKs (Hirayama et al. 1991): CDC2aAt is an A-type CDK which contains a PSTAIRE motif and CDC2bAt a B-type CDK which is plant specific and has a PPTALRE motif. The gene CDC2aAt is expressed constitutively throughout the cell cycle, whereas CDC2bAt is found to be preferentially expressed during S and G_2 (Segers et al. 1996). Seventeen cyclins have been identified in *A. thaliana* and also some other regulatory protein such as Cks1At (homologue of yeast Suc1/Cks1) and cyclin-dependent CDK kinase inhibitors have been described (Mironov et al. 1999).

There is an evolutionary conservation of *cdc2* genes, such that many *cdc2* yeast mutants can be rescued by their human or plant homologues.

A. thaliana CDC2aAt can complement yeast temperature-sensitive *S. pombe Cdc2* mutants or *S. cerevisiae CDC28* mutants, and restore cell division of these yeast strains at a restrictive temperature. More specifically, *CDC2aAt* can partially complement the *S. pombe cdc2-33*, a mutant whose single genetic lesion results in the twin defects of a loss of mitotic control and a loss of commitment to the cell cycle (Dickinson 1983).

It has been known for many years that herbicides belonging to different classes such as acetanilides and dinitroanilines are inhibitors of cell division (Kirkwood 1991).

The standard way to test the herbicidal activity of a compound or composition consists of spraying it on the whole plant or plant part or applying the compound to the soil prior to seedling emergence, and determining the herbicidal effect of the compound at specific time intervals after application. Such method requires a lot of space, can be expensive and time consuming. Much advantage could be gained by providing a relatively rapid and precise method for screening a wide variety of compounds for herbicidal activity. Such method would provide the means for large scale screening of a wide variety of test compounds.

It is thus an aim of the present invention to provide new methods for screening and identifying compounds or compositions which are useful as herbicides, plant growth regulators or fungicides.

It is further an aim of the present invention to provide new uses of yeast cells transformed with plant or animal cell cycle control genes.

It is further an aim of the present invention to provide new uses of recombinant vectors comprising plant or animal cell cycle control genes.

It is further an aim of the present invention to provide biological screening assays for identifying compounds or compositions which are useful as herbicides, plant growth regulators or fungicides.

It is also an aim of the present invention to provide compounds and compositions which act as herbicides, plant growth regulators or fungicides, as well as methods for producing the same.

It is also an aim of the present invention to provide a new use for known compounds or compositions as herbicides, plant growth regulators or fungicides.

A method for screening and identification of compounds or compositions useful as herbicides, growth regulators or fungicides involves at least the following steps:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of a yeast strain transformed with and expressing one or more plant cell cycle control genes or mutants thereof (phytoyeast) as well as to a control yeast strain; and

(b) determining the effect on the phenotype such as inhibition or stimulation of growth and/or cell division and/or changing cell shape/size of said phytoyeast compared to said control yeast.

In said method said plant polynucleic acids are expressed in yeast under the control of a repressible or inducible promoter, any other controlling element and/or culture conditions.

The screening method described in the present invention provides an efficient and rapid system for assaying the effect (inhibitory or stimulatory) of test compounds or compositions on the activity of plant proteins, in particular plant cell cycle control proteins, in yeast. Clearly, such compounds or compositions are candidates for the development of agrochemicals.

The present invention provides a yeast system that can serve as a screening tool for compounds that target the plant cell cycle control proteins and, thus, inhibit or activate the plant cell cycle.

The term "cell cycle" means the cyclic biochemical and structural events associated with growth and with division of cells, and in particular with the regulation of the replication of DNA and mitosis. Cell cycle includes phases called: G0, Gap1 (G1), DNA synthesis (S), Gap2 (G2), and mitosis (M). Normally these four phases occur sequentially however the cell cycle also includes modified cycles wherein one or more phases are absent resulting in modified cell cycle such as endomitosis, acytokinesis, polyploidy, polyteny, and endoreduplication.

Cell cycle control proteins and their role in regulating the cell cycle of eukaryotic organisms are reviewed in detail by John (1981) and the contributing papers therein (Norbury and Nurse 1992;Nurse 1990;Ormrod and Francis 1993) and the contributing papers therein (Doerner et al. 1996;Elledge 1996;Francis and Halford 1995;Francis et al. 1998;Hirt et al. 1991;Mironov et al. 1999) which are incorporated by reference.

The term "cell cycle control genes" refers to any gene or mutant thereof which exerts control on or are required for: chromosomal DNA synthesis and for mitosis (preprophase band, nuclear envelope, spindle formation, chromosome condensation, chromosome segregation, formation of new nuclei, formation of phragmoplast, etc) meiosis, cytokinesis, cell growth, endoreduplication, cell cycle control genes are also all genes exerting control on the above: homologues of CDKs, cyclins, E2Fs, Rb, CKI, Cks, and also any genes which interfere with the above, cyclin D, cdc25, Wee1, Nim1, MAP kinases, etc.

More specifically, cell cycle control genes are all genes involved in the control of entry and progression through S phase. They include, not exclusively, genes expressing "cell cycle control proteins" such as cyclin dependent kinases (CDK), cyclin dependent kinase inhibitors (CKI), D, E and A cyclins, E2F and DP transcription factors, pocket proteins, CDC7/DBF4 kinase, CDC6, MCM2-7, Orc proteins, cdc45, components of SCF ubiquitin ligase, PCNA, DNA-polymerase.

The term "cell cycle control protein" include cyclins A, B, C, D and E including CYCA1;1, CYCA2;1, CYCA3;1, CYCB;1, CYCB;2, CYC B2;2, CYCD1;1, CYCD2;1, CYCD3;1, and CYCD4;1 (Evans et al. 1983;Francis et al. 1998;Labbe et al. 1989;Murray and Kirschner 1989;Renaudin et al. 1996;Soni et al. 1995;Sorrell et al. 1999;Swenson et al. 1986) cyclin dependent kinase inhibitor (CKI) proteins such as ICK1 (Wang et al. 1997), FL39, FL66, FL67 (PCT/EP98/05895), Sic1, Far1, Rum1, p21, p27, p57, p16, p15, p18, p19 (Elledge 1996;Pines 1995), p14 and p14ARF; p13suc1 or CKS1At (De Veylder et al. 1997;Hayles and Nurse 1986) and nim-1 (Russell and Nurse 1987a;Russell and Nurse 1987b;Fantes 1989;Russell and Nurse 1986;Russell and Nurse 1987a;Russell and Nurse 1987b) homologues of Cdc2 such as Cdc2MsB (Hirt et al. 1993) CdcMs kinase (Bogre et al. 1997) cdc2 T14Y15 phosphatases such as Cdc25 protein phosphatase or p80cdc25 (Bell et al. 1993;Elledge 1996;Kumagai and Dunphy 1991;Russell and Nurse 1986) and Pyp3 (Elledge 1996) cdc2 protein kinase or p34cdc2 (Colasanti et al. 1991;Feiler and Jacobs 1990;Hirt et al. 1991;John et al. 1989;Lee and Nurse 1987;Nurse and Bissett 1981;Ormrod and Francis 1993) cdc2a protein kinase (Hemerly et al. 1993) cdc2 T14Y15 kinases such as wee1 or p107wee1 (Elledge 1996;Russell and Nurse 1986;Russell and Nurse 1987a;Russell and Nurse 1987b;Sun et al. 1999) mik1 (Lundgren et al. 1991) and myt1 (Elledge 1996); cdc2 T161 kinases such as Cak and Civ (Elledge 1996); cdc2 T161 phosphatases such as Kap1 (Elledge 1996);

cdc28 protein kinase or p34cdc28 (Nasmyth 1993;Reed et al. 1985) p40MO15 (Fesquet et al. 1993;Poon et al. 1993) chk1 kinase (Zeng et al. 1998) cds1 kinase (Zeng et al. 1998) growth-associated H1 kinase (Labbe et al. 1989;Lake and Salzman 1972;Langan 1978;Zeng et al. 1998) MAP kinases described by (Binarova et al. 1998;Bögre et al. 1999;Calderini et al. 1998;Wilson et al. 1999).

Other cell cycle control proteins that are involved in cyclin D-mediated entry of cells into G1 from G0 include pRb (Xie et al. 1996;Huntley et al. 1998) E2F, RIP, MCM7 and potentially the pRb-like proteins p107 and p130.

Other cell cycle control proteins that are involved in the formation of a pre-replicative complex at one or more origins of replication, such as, but not limited to, ORC, CDC6, CDC14, RPA and MCM proteins or in the regulation of formation of this pre-replicative complex, such as, but not limited to, the CDC7, DBF4 and MBF proteins.

For the present purpose, the term "cell cycle control protein" shall further be taken to include any one or more of those proteins that are involved in the turnover of any other cell cycle control protein, or in regulating the half-life of said other cell cycle control protein. The term "protein turnover" is to include all biochemical modifications of a protein leading to the physical or functional removal of said protein. Although not limited to these, examples of such modifications are phosphorylation, ubiquitination and proteolysis. Particularly preferred proteins which are involved in the proteolysis of one or more of any other of the above-mentioned cell cycle control proteins include the yeast-derived and animal-derived proteins, Skp1, Skp2, Rub1, Cdc20, cullins, CDC23, CDC27, CDC16, and plant-derived homologues thereof (Cohen-Fix and Koshland 1997;Hochstrasser 1998;Krek 1998;Lisztwan et al. 1998) and Plesse et al in (Francis et al. 1998)).

For the present purpose, the term "cell cycle control genes" shall further be taken to include any one or more of those gene that are involved in the transcriptional regulation of cell cycle control gene expression such as transcription factors and upstream signal proteins. Additional cell cycle control genes are not excluded.

For the present purpose, the term "cell cycle control genes" shall further be taken to include any plant cell cycle control genes or mutant thereof, which are affected by environmental signals such as for instance stress, nutrients, pathogens, or by intrinsic signals such as the plant hormones (auxins, cytokinins, ethylene, gibberellic acid, abscisic acid and brassinosteroids).

For the present purpose, the term "cell cycle control genes" shall further be taken to include any plant cell cycle control genes or a mutant thereof which when expressed in yeast affect cell division positively or negatively, through interactions with the endogenous yeast cell cycle machinery.

A preferred embodiment of the invention relates to any plant cell cycle control gene or mutant thereof complementing a mutation in a yeast homologue such as plant genes encoding cyclin-dependent kinases (e.g. A type CDK), cyclins (e.g. cyclin D (complement triple CLN mutation (Francis and Halford 1995)), or CKS. Also the method comprises any plant cell cycle control genes or mutants thereof which have no homologue in yeast but which interfere with the endogenous cell cycle of the host yeast through interaction with the cyclin-dependent kinases, e.g. the plant cyclin-dependent kinase inhibitors (CKIs).

The present invention encompasses the use of cell cycle control genes coding for cell cycle control proteins selected from the examples described above, such genes including sense, antisense, dominant negative, wild-type or mutant versions thereof and any functional homologous gene related thereto.

The present invention clearly encompasses the use of homologues, analogues or derivatives of any of the above mentioned cell cycle control proteins which function in DNA synthesis, mitosis, S phase, endomitosis, acytokinesis, polyploidy, polyteny, and endoreduplication.

Preferably, the cell cycle control gene is derived from a yeast or plant cell or animal cell, more preferably, from a plant cell, such as a monocotyledonous or dicotyledonous plant cell (Mironov et al. 1999), more preferably from *A. thaliana* or *Oryza sativa.*

Expression of *A. thaliana* cell cycle control genes or mutants thereof in temperature-sensitive Cdc2-mutant yeast provides a useful model for identifying novel compounds or compositions.

Yeast cells are useful to the present invention as they present the advantage of being simple eukaryotes, inexpensive and safe to grow. The yeast also have short generation times, simplicity of nutritional requirements, rapid growth and ease of cultivation and preservation. They are easy to analyse and transform using classical and molecular techniques know by the skilled in the art.

More specifically, the present invention utilises a genetically engineered organism that expresses plant cell cycle control genes or mutants thereof. The invention also

features a host transformed with an exogenous DNA molecule comprising plant cell cycle control genes or mutants thereof. The transformed host can be for example any strain of *Schizosaccharomyces*, *Saccharomyces* or *Candida*. More specifically, the present invention utilises a genetically engineered strains of the yeast *Schizosaccharomyces pombe* or *Saccharomyces cerevisiae,* which expresses plant cell cycle control genes or mutants thereof from any plant, such as *Arabidopsis thaliana* (thale cress) and *Oryza sativa* (rice). More in general, it can be any monocotyledonous or dicotyledonous plant, preferably they belong to a plant species of interest in agriculture, wood culture or horticulture, such as a crop plant, root plant, oil producing plant, wood producing plant, agricultured plant, fruit-producing plant, fodder or forage legume, companion plant, or horticultured plant. Such plants are e.g. apricot, artichoke, asparagus, apple, banana, barley, broccoli, Brussels sprouts, cabbage, canola, carrot, cassava, cauliflower, celery, cherry, chicory, collard greens, cotton, Douglas fir, fir (*Abies* and *Picea* species), flax, garlic, grapes, kale, lentil, maize, oak, oats, oilseed rape, okra, onion, pear, pepper, poplar, potato, rye, sorghum, soybean, squash, sugar beet, sugar cane, sunflower, tobacco, tomato, wheat. Additional species are not excluded. In particular genes from weed species are included. In particular, the invention provides a method to screen for compounds or compositions, which inhibit or activate plant cell division using mutant yeast strains containing one or more plant cell cycle control genes. The screens can also be used to identify compounds that inhibit yeast cell cycle control proteins without affecting their plant homologues.

The present invention comprises the use a transformed yeast strain (phytoyeast) containing a plant cell cycle control gene or mutant thereof which is functionally complementing an essential endogenous cell cycle control gene or mutant thereof. The method involves at least the steps of:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of said phytoyeast expressing said plant cell cycle control protein or a mutant thereof, as well as to a control yeast strain;

(b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast;

(c) identifying a compound or composition which affects said phenotype of said phytoyeast but not of said control yeast; and

(d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

The present invention also encompasses a method wherein a transformed yeast strain (phytoyeast) expresses a plant cell cycle control gene or dominant mutant thereof and wherein said method involves at least the following steps:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of said phytoyeast expressing said plant cell cycle control gene or dominant mutant thereof as well as to a control yeast strain;

(b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast;

(c) identifying a compound or composition which affects said phenotype of said phytoyeast but not of said control yeast; and

(d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

In one embodiment of the present invention, said transformed yeast (phytoyeast) expresses a plant cell cycle control gene which does not complement endogenous yeast cell cycle control genes but which affects the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast.

In another embodiment of the present invention, said transformed yeast (phytoyeast) expresses a plant cell cycle control gene with a dominant negative mutation resulting in growth arrest.

In yet another embodiment of the present invention, said transformed yeast (phytoyeast) expresses a plant cell cycle control gene with a dominant positive mutation resulting in growth acceleration.

The present invention further comprises a method wherein a transformed yeast (phytoyeast) expresses a first plant cell cycle control gene or mutant thereof, with the activity of its encoded protein being modulated by the expression of a second plant cell cycle control gene. Said method encompasses the following steps:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of a strain of said double transformed yeast (phytoyeast) expressing at least said first and said second plant cell cycle control gene or mutants thereof as well as to said phytoyeast only expressing said first plant cell cycle control gene;

(b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said double transformed phytoyeast expressing at least said first and said second plant cell cycle control gene compared to said phytoyeast only expressing said first plant cell cycle control gene;

(c) identifying a compound or composition which affects said phenotype of said double transformed phytoyeast expressing at least said first and said second plant cell cycle control gene compared to said phytoyeast only expressing said first plant cell cycle control gene; and

(d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

In a preferred embodiment of the present invention, said first plant cell division cycle control protein is encoded by the *A. thaliana cdc2a* gene or mutant thereof and said second plant cell cycle control protein or mutant thereof is encoded by another cell cycle control gene or mutant thereof, more preferably an *A. thaliana* CKI or mutant thereof.

The present invention also involves the addition of the compound or composition to be screened to a culture of a genetically engineered organism capable of expressing plant genes or mutants thereof and the determination of whether the organism's phenotype (growth and/or cell division and/or cell size/shape) is affected.

The present invention more particularly comprises the utilisation of yeast in which one or more plant cell cycle control genes or mutants thereof functionally complement one or more essential endogenous cell cycle control gene. The invention also employs yeast that expresses plant cell cycle control genes or dominant mutations thereof (Hemerly et al.1995, Porceddu et al. 1999). Furthermore, the invention comprises the use of yeast in which a plant cell cycle control gene or mutant thereof is expressed, which activity is regulated by the co-expression of another plant cell cycle control gene or mutant thereof.

In another embodiment of the present invention, said transformed yeast express the CAK1At plant gene or mutant thereof from *Arabidopsis thaliana* or the R2 gene or mutant thereof from *Oryza sativa* both genes functionally complementing the CAK1 gene from *S. cerevisiae*.

This screening method encompasses a method where test compounds or compositions are added to a culture or a culture area of a strain of transformed yeast expressing plant cell cycle control genes or mutants thereof. The test compounds

and yeast are incubated together and the effects of said compounds or compositions on yeast phenotype (growth and/or cell division and/or cell size/shape) are measured. The effects of compounds or compositions on the plant cell cycle control proteins can be evaluated by means of the assay on the phytoyeast. The use of suitable control yeast allows to distinguish differential action of compounds or compositions on plant cell cycle control protein as opposed to the equivalent yeast or animal or human cell cycle control protein. Figure 1 shows a flow chart giving an overview of the phytoyeast screening assay.

The genetically engineered yeast can be used for rapid screening of chemical compounds or compositions that have an activity on the plant cell cycle by measuring a compound's ability to alter the phenotype (growth and/or cell division and/or cell size/shape) of the mutant yeast cells. These compounds or compositions may have an activity as herbicides or plant growth regulators or fungicides. The present invention comprises also a biological assay, as the active compounds or compositions are able to enter into living cells.

The present invention allows high throughput screening of chemicals to determine active compounds. This invention is based upon the finding that chemical agents of potential value as herbicides or growth regulators can be identified using a yeast mutant assay expressing plant cell cycle control genes or mutants thereof.

The yeast cell cycle control genes may be replaced by homologous plant cell cycle control genes or mutants thereof able to function in the yeast environment.

According to one embodiment of the present invention a method of screening for test compounds which have an effect on the growth of "phytoyeast " is provided. The effect of the test compound on the plant cell cycle is deduced from the experiment. This method comprises functionally complementing a yeast cell cycle control gene by a plant cell cycle control gene or mutant thereof (e.g. *S. pombe* cdc2 complemented by *A. thaliana* CDC2aAt (Hirayama et al. 1991); the *S. pombe* SUC1 complemented by the *A. thaliana* CKS1, (Porceddu et al. 1999), the *S. cerevisiae* CAK1 complemented by the *A. thaliana* CAK1At (Umeda et al, 1998) or the R2 gene from *O. sativa* (Yamagushi et al, 1998)). The yeast will only grow when the plant cell cycle control gene is expressed and test compounds can be screened that alter the phenotype (growth and/or cell division and/or cell size/shape) of the phytoyeast but not the yeast which contains the wild-type yeast gene or yeast in which the wild-type gene is replaced by an animal or human homologue of the plant cell cycle control

gene (e.g. human CDC2, human CKS) (assessing selectivity of the compound). Conversely, compounds which inhibit growth of the wild type but not of the transformed yeast (phytoyeast) will be suitable for use as fungicides.

According to another embodiment, the present invention provides a method for identifying the effect of test compounds on the phenotype (growth and/or cell division and/or cell size/shape) of a transformed yeast (phytoyeast). The method comprises the use of plant cell cycle control genes or dominant mutations thereof resulting in altered phenotype in the transformed yeast (phytoyeast) and of yeast expressing these genes under the control of a regulatory element. The screening method aims at identifying compounds or compositions restoring the wild-type yeast phenotype. An exemplary dominant negative mutant is a gene encoding the *Arabidopsis* CDC2aAt.DN protein in which the D146 residue is substituted for the N146 residue, an example of a dominant positive mutant is a gene encoding the Arabidopsis CDC2aAt.AF protein in which the T14Y15 residues are substituted for the A14F15 residues.

According to yet another embodiment in the present invention is provided a screen comprising yeast in which a first plant cell cycle control gene or mutant thereof is expressed and with the activity of its encoded protein being modulated by the expression of another (second) plant cell cycle control gene or mutant thereof under the control of an inducible promoter. Compounds or compositions are identified which revert the phenotype of a phytoyeast expressing the two plant cell cycle control genes to the phenotype of said transformed yeast (phytoyeast) when only expressing the first plant cell cycle control protein. An exemplary first plant cell cycle control protein is a dominant positive mutant of CDC2aAt (CDC2a.AF; causes wee-phenotype when expressed in yeast) and an exemplary second plant cell cycle control protein is A. thaliana CKI (when co-expressed with CDC2a.AF, elongated yeast cells are observed). Compounds which interfere with the CDC2a.AF-CKI interaction and revert the elongated yeast cells to the wee-phenotype are thus identified.

The present invention also comprises a method to select plant specific compounds by screening for compounds which affect the phenotype (growth and/or cell division and/or cell shape/size) of the transformed yeast (phytoyeast) and not of yeast mutants expressing animal or human homologues of the plant cell cycle control genes expressed in the phytoyeast (Paris et al. 1994). Conversely, compounds

which inhibit growth of the wild type but not of the mutant yeast (phytoyeasts and yeast mutants expressing animal or human cell cycle control genes) will be suitable for use as fungicides.

The present invention includes the use of a recombinant vector comprising at least one polynucleic acid encoding at least part of a plant cell cycle control protein or a mutant thereof to transform yeast for the screening or identification of compounds or compositions which abolish, retard or stimulate plant growth. Said recombinant vector is a plasmid, more particularly a vector comprising a selectable marker and transcriptional control elements for the expression of said plant or animal/human cell cycle control polynucleic acids in yeast. Said plant or animal/human cell cycle control polynucleic acid is integrated into the yeast genome by random, non-homologous or homologous recombination.

The plant genes referred to in the present invention can be inserted into yeast by means of autonomously replicating plasmids, thus propagating as extrachromosomal elements. These vector plasmids, known as shuttle-vectors, consist of a bacterial origin of replication, an autonomous replication sequence (ars) and a selectable marker (Fission Yeast Handbook). Any selectable marker can be used. Markers usually utilised in *S. pombe* are *ura4*$^+$, *leu1*$^+$, *his3*$^+$ and *sup3-5*, and preferably leu1+ (Hemerly et al. 1995). Other controlling elements such as promoter sequences and transcription termination sequences are included for expression of the plant genes.

Alternatively, the plant genes may be integrated into the yeast genome. This may be done by random, non-homologous recombination or by homologous recombination directed by cloned yeast sequences into a predetermined site in the chromosome. Controlling elements like those used in plasmid vectors would regulate expression of the plant genes. Different promoter sequences may be used to vary the level of expression of the plant gene product (De Veylder et al. 1997).

Expression vectors may be introduced into host cells by various methods known in the art. The yeast transformation may be done using different procedures, comprising the lithium acetate method, the electroporation method, the protoplast method or the lithium chloride method. In a preferred embodiment of the invention the lithium acetate procedure is used (Current Protocols in Molecular Biology; Fission Yeast Handbook).

These genes, cDNAs or synthetically produced coding sequences can be cloned in pREP-type extrachromosomally replicating plasmids or pRIP-type integrative

expression plasmids, under the control of a suitable repressible promoter preferably thiamine repressible promoter. These plasmids permit thiamine-mediated control of transcription to be applied to the cloned gene (Maundrell 1993).

More specifically the wild type and mutated cell cycle control cDNAs from *A. thaliana* might be cloned in pREP3 or pREP41 or pREP81 expression vector containing LEU2 as a selectable marker and under the control of the thiamine-repressible promoter *nmt1* (Maundrell, 1993). The constructs can be transformed in *S. pombe* using the lithium acetate method.

The yeast employed according to the present invention may be any strain of *Schizosaccharomyces* species (e.g. *S. pombe)*, *Saccharomyces* species (e.g. *S. cerevisiae)*, or *Candida* species (e.g. *C. albicans*). In a particularly preferred embodiment of the present invention, the species *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are employed as their cell cycle machinery has greater homologies with the higher eukaryotic systems, including their post-translational protein modification mechanisms. Furthermore the fission yeast cells grow rapidly on simple media, have a regular size and cell division cycle mutants exhibit specific phenotypes.

The term "compound or compositions" according to the present invention refers to a chemical or a mixture of chemicals identified by the methods described herein which inhibit or activate either directly or indirectly one or more plant cell cycle pathways. The compound or compositions to be identified for interference with the plant cell cycle are introduced in the cell culture medium for example.

The present invention more particularly relates to a method for identifying a compound or composition which comprises solubilising said test compound or composition in a solvent, diluting it and adding it to the cell culture medium of said yeast strains. Said compound or composition can be added to a disk or to a well on a culture plate, in a standard diffusion assay using solidified media, or can be added directly into liquid media. Said yeast are exposed to a range of concentrations of said test compound or composition. The determination of the effect on the phenotype (growth and/or cell division and/or cell size/shape) consists of measuring the decrease or increase of the degree of growth and/or cell division and/or analysing the phenotype of said phytoyeast and said control yeast upon addition of said compound or composition. Transformed yeast cells expressing at least one plant cell

cycle control gene or a mutant thereof are used for the screening or identification of compounds or compositions which abolish, retard or stimulate plant growth.

In a method according to the present invention, a test compound or compositions can be introduced into a culture or a culture area of mutant yeast that expresses plant cell cycle control genes or mutants thereof.

The yeast transformed with a gene, cDNA or synthetically produced coding sequence encoding plant cell cycle control proteins or mutants thereof is allowed to grow under suitable conditions. Growth of the host cells may be achieved by one of ordinary skill in the art by the use of an appropriate medium. Appropriate media for growing host cells include those providing nutrients necessary for the growth of the cells. A typical medium for growth includes necessary carbon sources, nitrogen sources and trace elements. Inducers may also be added. Methodologies for selective culturing conditions suitable for a variety of transformed yeast may be found in Current Protocols in Molecular Biology, and the Fission Yeast Handbook.

The methods according to the present invention comprise solubilising the test compound or composition in a solvent such as DMSO, diluting it and adding it to the cell culture medium, or to a buffer. The test compound or composition can also be added to a disk or to a well on a culture plate, in a standard diffusion assay using solidified media, or the preparation can be introduced directly into liquid media. The preparation is added directly to the culture medium surrounding the phytoyeast or into the phytoyeast. The phytoyeast capable of expressing plant cell cycle control genes or mutants thereof and the control yeast can be exposed to a single concentration, or to a range of concentrations of a test compound or composition. If necessary the culture conditions can be altered, resulting in restrictive growth conditions or inducing growth conditions. The control yeast and phytoyeast cell cultures are incubated with the test compound.

The effects of the test compound or compositions on the control yeast and phytoyeast are determined. The presence or absence and sometimes the degree of growth are measured and the phenotype (growth and/or cell division and/or cell size/shape) is analysed. The effect on the growth of the phytoyeast exposed to the test compound or compositions is compared to the control yeast (wild type yeast, phytoyeast or yeast expressing animal/human genes) which has been incubated without the test compound. The effects of test compound or compositions on the growth of the phytoyeast are also compared to the effects of said compound or

compositions on the wild type yeast or yeast expressing animal/human genes, to determine if the compound has a specific action on the plant cell cycle control proteins.

Effect of test compounds or compositions on the phytoyeast can be measured by means known to those skilled in the art and include evaluation of the mutant yeast phenotype, shape, size, number, growth rate, growth stimulation or growth inhibition, and comparison with the wild type. The effect on the plant cell cycle control genes can readily be deduced from these studies. Subsequently the effective concentration of the test compound on the phytoyeast can be further evaluated.

The present invention provides a method easily adaptable to microtiter plate technology, providing a rapid and inexpensive method. Cell growth and inhibition as determined by turbidity can also be measured by standard spectrophotometric instrumentation.

The test compounds or compositions that have been identified according to the invention as inhibitor or activation of the plant cell cycle, are further evaluated by testing the compound on whole plants in standard herbicidal greenhouse tests well known to those skilled in the art (Fedtke 1982).

The compounds or compositions identified as having an effect on the plant cell cycle, may be manufactured or used in the preparation of herbicides or plant growth regulators. Furthermore, the compounds or compositions identified as having an effect on the cell cycle may be further tested for likely therapeutical utility. Furthermore, compounds that have been identified according to the invention as inhibitor or activator of the yeast cell cycle may be used in the preparation of potential fungicides.

Additionally, the present invention extends to a substance identified by a method according to the invention as an inhibitor or activator of the plant cell cycle, for use as herbicides or plant growth regulators or the use of such substance in the preparation of pesticides.

The method of the invention presents numerous advantages, including speed and simplicity, thus a large number of test compounds can be tested rapidly and inexpensively. In particular the use of yeast cells renders the methods described herein easy to perform and compatible with high throughput screening procedures.

The present invention also encompasses a biological screening assay comprising the use of transformed yeast (phytoyeast) expressing plant cell cycle control proteins

to identify novel compounds or compositions that affect control yeast or phytoyeast phenotype. Said screening assay is used for identifying compounds or compositions which act as herbicides, plant growth regulators or fungicides.

The present invention further includes a high throughput screening system to determine compounds or compositions which act as herbicides, plant growth regulators or fungicides involving said method, use or biological assay, preferably a microtiter plate screening system.

The present invention furthermore encompasses compounds or compositions identifiable by any said method.

A method for producing a pesticide, herbicide, plant growth regulator or fungicide, is described comprising the steps of:

(a)identifying a compound or composition or a derivative or homologue thereof, and,

(b)mixing said compound, composition, derivative or homologue thereof with an acceptable carrier.

Compounds or compositions as defined above can be used for inhibiting or stimulating plant growth and/or for increasing crop yield and/or for preventive or curative protection of the plant against fungal infection.

The present invention also comprises a yeast strain expressing CDC2a.AF and a cyclin-dependent kinase inhibitor of *A. thaliana* resulting in an elongated cell type.

The present invention describes a compound screening assay comprising the use of said yeast strain.

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

EXAMPLES

Example 1. Determination of the biological activity of mutant CDC2a proteins in fission yeast and use of the yeast mutants in a screening assay

Based on dominant mutations described in yeast, two mutant *Arabidopsis* Cdc2a cDNAs are constructed. The first mutant is a CDC2aAt protein with substitution of the D146 residue for the N146 residue. The second mutant is a CDC2aAt protein with substitutions of the T14 and Y15 residues, for A14 and F15, respectively.

Site-directed mutagenesis and plasmid transformation were performed as described (Hemerly et al. 1995), (Porceddu et al. 1999)

The CDC2a cDNAs and its mutant forms were cloned in the *S. pombe* expression vector pREP3 containing LEU2 as a selectable marker, under the control of the thiamine-repressible promoter nmt1. The plasmid constructs are transformed into the cdc2-33 leu1-32h$^-$ fission yeast strain by the lithium acetate method

When expressed in yeast, CDC2a.DN generated cells with an elongated shape, typical of cell cycle arrest (Cdc phenotype). The expression in yeast of the second mutant CDC2a.AF causes the cells to enter into premature mitosis, with cells that are smaller than wild type cells (wee phenotype). Many test compounds and composition are screened using the mutant yeast.

The test compounds are dissolved in methanol then diluted and added on disk to the growth medium by a standard diffusion assay. The wild type yeast and the yeast mutants (phytoyeast) are grown on media with and without added test compound. The latter cultures serve as controls against which the effect of the test compound on the heterologous cell cycle control protein(s) can be assessed.

CDC2aDN mutant yeast cells present a Cdc phenotype. Test compounds are identified which restore cell division of the mutant yeast. Test compounds are also identified which inhibit the uncontrolled cell division of CDC2aAF mutant. Compounds interfering with cell cycle control genes are thus identified.

Example 2. CKS1At gene of Arabidopsis thaliana is capable of rescuing a temperature sensitive S. pombe cdc2 allele.

The suc1 gene of *Schizosaccharomyces pombe* was originally identified as a suppressor of certain temperature-sensitive alleles of cdc2 (Hayles et al. 1986). The *Arabidopsis thaliana* CKS1At gene encodes a functional homologue of the *S. pombe*

SUC1 protein and is capable of rescuing temperature-sensitive fission yeast cdc2 allele.

The CKS1At gene is cloned in different pREP expression vectors under the control of the *nmt1* promoter, allowing the inducible expression of the CKS1At to different levels. The plasmid constructs are transformed into cdc2-L7 fission yeast strain by the lithium acetate method.

Low to moderate expression levels of the CKS1At gene allowed the temperature-sensitive cdc2-L7 strain to divide at restrictive temperature, suggesting that CKS1At can substitute for the fission yeast SUC1.

Yeast expressing CKS1At are used to screen test compounds. As previously described the test compounds are prepared and added to the culture medium in a standard diffusion assay. Wild type and yeast expressing CKS1At are grown at restrictive temperature with and without the test compounds. Compounds which inhibit the growth of the phytoyeast and not the wild type yeast are identified as potential herbicides.

Example 3. Conversion of a dominant positive form of CDC2aAt into a negative one by co-expression of the CKI genes, and screening for compounds capable of interfering with CDC2aAT-CKI interactions.

The ability to convert a dominant positive form of the *Arabidopsis thaliana* CDC2aAt protein into a negative form using yeast cells as host was tested by the use of the recently isolated cyclin-dependent kinase inhibitors (CKIs) of *Arabidopsis thaliana*. These CKIs were isolated using the CDC2aAt protein as a bait in a two-hybrid screen, and were shown to possess in vitro CDK inhibitory activity (Wang et al. 1997). The full length coding regions of the CKIs were cloned in the pREP41HA vector (Craven et al. 1998) under the control of the attenuated *nmt1* promoter. This construct allows inducible expression of the transgene to relative moderate expression levels when cells are grown in thiamine-free medium. In this vector the CKI genes are cloned in fusion to the haemaglutinin (HA) tag, allowing to detect the induced protein in crude protein extracts by western blotting using a HA-specific antibody.

The pREP41HA/CKI constructs were transformed into the leu1-32 ura4-D18 h⁻ fission yeast strain in combination with the pREP1/CDC2a.AF or the control pREP1 vector. pREP1/CDC2a.AF allows relative strong induction of a mutant CDC2aAt

protein with substitutions of the T14 and Y15 residues, for A14 and F15, respectively. When expressed in yeast, CDC2a.AF causes a raise in the total CDK activity of the cell, pushing the cells into a premature mitosis (Porceddu et al. 1999). This can be observed as the appearance of cells that are smaller than wild type cells.

Transformation of yeast cells with the pREP41HA/CKI constructs in combination with the control pREP41 vector did not affect the yeast cell cycle when cells were grown in inducing medium, as can be seen from their normal growth and cell size. This is not unexpected, as the plant CKIs do not show any sequence identity with the yeast CKIs. Surprisingly, when the plant CKIs were expressed in the yeast cells in combination with CDC2a.AF, long elongated cells could be observed, instead of the small cells that can be seen when only CDC2a.AF is expressed. This phenotype can be explained by assuming that the CDC2a.AF protein is still able to interact with some of the yeast cell cycle control proteins, thereby titrating them out of the wild type yeast CDK protein. When only CDC2a.AF is present in the yeast cell, it will form an active complex with the yeast proteins, driving cell division. However, in the additional presence of the plant CKIs, as demonstrated by western blotting, the activity of CDC2a.AF is inhibited, causing a cell cycle arrest. Test compounds are screened which are capable of inducing a change in the phenotype of the double transformant, from elongated cells (cell cycle arrest, Cdc phenotype) to small dividing cells (wee phenotype).

The test compounds are dissolved in DMSO then diluted and added to the growth medium by mixing the compound into the medium. The wild type and yeast double transformant are incubated with and without the test compounds, and active compounds are identified which induce a change in the phenotype of the double transformant therefore interfering with the CDC2a.AF-CKI complex.

Example 4. Complementation of Saccharomyces cerevisiae CAK1 by CAK1At from *Arabidopsis thaliana*, and by the R2 gene *from Oryza sativa*.

The activation of the CDKs is mediated by phosphorylation process as well as by binding to cyclins. CDK-activating kinases (CAKs) catalyse the phosphorylation of a threonine residue within the T-loop of CDKs. The R2 protein from Oryza sativa is very similar to CAKs of metazoan and fission yeast and was shown to suppress a CAK1/CIV1 mutation in budding yeast (Yamagushi et al, 1998). While having no

significant sequence similarity to CAK1/CIVI from *S. cereivisiae*, CAK1At from *Arabidopsis thaliana* when overexpressed in yeast was capable of rescuing temperature sensitive mutant yeast cells (mutation in the CAK1/CIVI gene) (Umeda et al, 1998). Two phytoyeast one expressing *A. thaliana* CAK1At gene and the other one expressing the R2 gene from *O. sativa* are contructed.

The CAK1At cassette and the *O. sativa* R2 cassette are PCR amplified and introduced into yeast using the Lithium acetate transformation method. The endogenous CAK1/CIVI gene from cerevisiae is replaced respectively by the CAK1At gene or *the O. sativa* R2 gene by homologous recombination using a PCR-based approach with long tracts of flanking homology. The transformants were screened for homologous recombination by PCR. The transformed yeast expressing *A. thaliana* CAK1At, and the yeast expressing *O.sativa* R2 gene are used and compared against wild type yeast for compounds testing.

Different concentration of the test compounds are dissolved in methanol then diluted and added to the growth medium by mixing the compound into the medium. The phytoyeast and the wild type are incubated with and without the test compounds, and active compounds are identified which inhibit the growth of the phytoyeast and not the wild type suggesting an specific interaction with the plant gene product.

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CLAIMS

1. A method for screening and identification of compounds or compositions useful as herbicides, growth regulators or fungicides involving at least the following steps:
   (a) addition of the compound or composition to be screened or identified to a culture or culture area of a yeast strain transformed with and expressing one or more plant cell cycle control genes or mutants thereof (phytoyeast) as well as to a control yeast strain; and,
   (b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast.

2. The method of claim 1, wherein said plant polynucleic acids are expressed in yeast under the control of a repressible or inducible promoter, any other controlling element and/or culture conditions.

3. The method of claim 1 or 2 wherein said yeast is any strain of *Saccharomyces cerevisiae*, *Candida albicans* or more preferably *Schizosaccharomyces pombe*.

4. The method of any of claims 1 to 3, wherein said transformed yeast strain (phytoyeast) contains a plant cell cycle control gene or mutant thereof which is functionally complementing an essential endogenous cell cycle control gene or mutant thereof, involving at least the steps of:
   (a) addition of the compound or composition to be screened or identified to a culture or culture area of said phytoyeast expressing said plant cell cycle control protein or a mutant thereof, as well as to a control yeast strain;
   (b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast;
   (c) identifying a compound or composition which affects said phenotype of said phytoyeast but not of said control yeast; and,
   (d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

5. The method of any of claims 1 to 3, wherein said transformed yeast strain (phytoyeast) expresses a plant cell cycle control gene or dominant mutant thereof, wherein said method involves at least the following steps:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of said phytoyeast expressing said plant cell cycle control gene or dominant mutant thereof as well as to a control yeast strain;

(b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast compared to said control yeast;

(c) identifying a compound or composition which affects said phenotype of said phytoyeast but not of said control yeast; and

(d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

6. The method of claim 5, wherein said transformed yeast (phytoyeast) expresses a plant cell cycle control gene which does not complement endogenous yeast cell cycle control genes but which affects the phenotype (growth and/or cell division and/or cell size/shape) of said phytoyeast.

7. The method of claim 5, wherein said transformed yeast (phytoyeast) expresses a plant cell cycle control gene with a dominant negative mutation resulting in growth arrest.

8. The method of claim 5, wherein said transformed yeast (phytoyeast) expresses a plant cell cycle control gene with a dominant positive mutation resulting in growth acceleration.

9. The method of any of claims 1 to 3, wherein said transformed yeast (phytoyeast) expresses a first plant cell cycle control gene or mutant thereof with the activity of its encoded protein being modulated by the expression of a second plant cell cycle control gene or mutant thereof, wherein said method comprises the following steps:

(a) addition of the compound or composition to be screened or identified to a culture or culture area of a strain of said double transformed yeast (phytoyeast) expressing at least said first and said second plant cell cycle control gene or mutants thereof as well as to said phytoyeast only expressing said first plant cell cycle control gene;

(b) determining the effect on the phenotype (growth and/or cell division and/or cell size/shape) of said double transformed phytoyeast expressing at least said first and said second plant cell cycle control gene compared to said phytoyeast only expressing said first plant cell cycle control gene;

(c) identifying a compound or composition which affects said phenotype of said double transformed phytoyeast expressing at least said first and said second plant cell cycle control gene compared to said phytoyeast only expressing said first plant cell cycle control gene; and

(d) confirming whether said compound or composition also inhibits or stimulates the growth of plants.

10. The method of claim 9, wherein said first plant cell division cycle control protein is preferably encoded by the *A. thaliana cdc2a* gene or mutant thereof and said second plant cell cycle control protein or mutant thereof is encoded by another cell cycle control gene or mutant thereof, preferably an *A. thaliana* CKI or mutant thereof.

11. A method for identifying plant specific compounds or compositions comprising the steps of a method of any of claims 1 to 10 wherein said compound or composition affects the phenotype (growth and/or cell division and/or cell shape/size) of a yeast strain transformed with one or more plant cell cycle control genes (phytoyeast) and not of a wild type control yeast and not of a control yeast expressing an animal or human homologue(s) of the plant cell cycle control gene(s) expressed in the phytoyeast.

12. A method for identifying fungicide compounds or compositions comprising the steps of a method of claim 1 to 10 wherein said compound or composition inhibits growth of the wild type control yeast but not of the mutant control yeast being either a phytoyeast and/or a yeast expressing the corresponding animal or human cell cycle control gene.

13. The method for identifying a compound or composition of any of claims 1 to 12, comprising solubilising said test compound or composition in a solvent, diluting it and adding it to the cell culture medium of said yeast strains.

14. The method of any of claims 1 to 13, wherein said compound or composition is added to a disk or to a well on a culture plate, in a standard diffusion assay using solidified media, or is added directly into liquid media.

15. The method of any of claims 1 to 14, wherein said yeast are exposed to a range of concentrations of said test compound or composition.

16. A method of any of claims 1 to 15, wherein the determination of the effect on the phenotype (growth and/or cell division and/or cell size/shape) consists of measuring the decrease or increase of the degree of growth and/or cell division and/or analyzing the phenotype of said phytoyeast and said control yeast upon addition of said compound or composition.

17. Use of transformed yeast cells expressing at least one plant cell cycle control gene or a mutant thereof as defined in any of claims 1 to 3 for the screening or identification of compounds or compositions which abolish, retard or stimulate plant growth.

18. Use of a recombinant vector comprising at least one polynucleic acid encoding at least part of a plant cell cycle control protein or a mutant thereof as defined in any of claims 1 to 3 to transform yeast for the screening or identification of compounds or compositions which abolish, retard or stimulate plant growth.

19. The use of claim 18, wherein said recombinant vector is a plasmid, more particularly a vector comprising a selectable marker and transcriptional control elements for the expression of said plant or animal/human cell cycle control polynucleic acids in yeast.

20. The method or use of any of claims 1 to 19, wherein said plant or animal cell cycle control polynucleic acid is integrated into the yeast genome by random, non-homologous or homologous recombination.

21. A biological screening assay comprising the use of transformed yeast (phytoyeast) as defined in any of claims 1 to 20 expressing plant cell cycle control proteins to identify novel compounds or compositions that affect control yeast or phytoyeast phenotype.

22. The use of a screening assay according to claim 21 for identifying compounds or compositions which act as herbicides, plant growth regulators or fungicides.

23. A high throughput screening system to determine compounds or compositions which act as herbicides, plant growth regulators or fungicides involving a method, use or biological assay of any of claims 1 to 22, preferably a microtiter plate screening system.

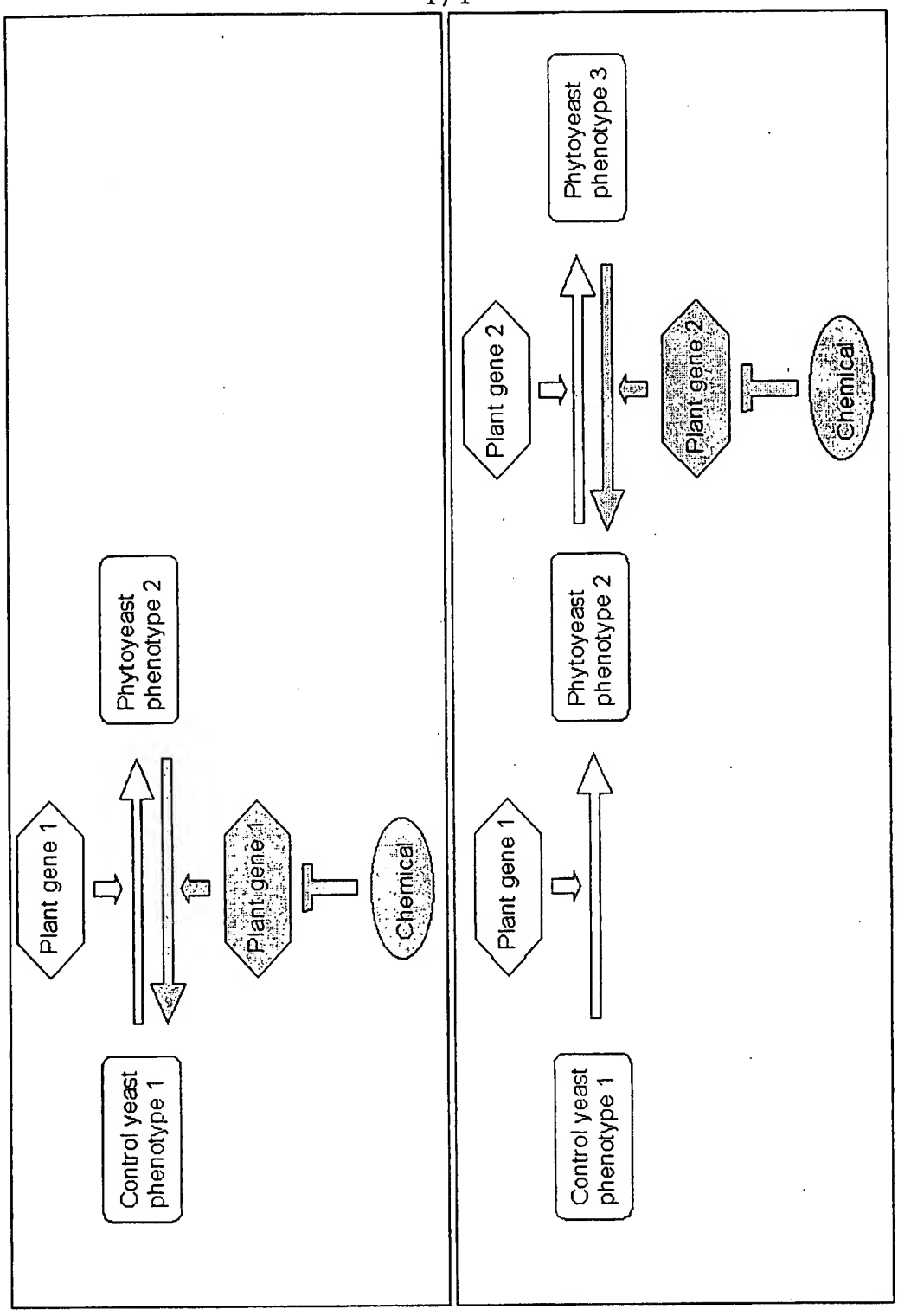
24. A compound or composition identifiable by a method according to any of claims 1 to 23.

25. A method for producing a pesticide, herbicide, plant growth regulator or fungicide, comprising the steps of:
    (a)identifying a compound or composition as defined in any of claims 1 to 24 or a derivative or homologue thereof, and,
    (b)mixing said compound, composition, derivative or homologue thereof with an acceptable carrier.

26. Use of a compound or composition according to claim 24 in a method according to claim 25 for inhibiting or stimulating plant growth and/or for increasing crop yield and/or for preventive or curative protection of the plant against fungal infection.

27. A yeast strain expressing CDC2a.AF and a cyclin-dependent kinase inhibitor of *A. thaliana* resulting in an elongated cell type.

28. A compound screening assay comprising the use of a yeast strain of claim 27.



Overview of the phytoyeast screening strategy

Phenotype : Growth and/or absence of growth and/or cell division and/or cell size/shape

FIGURE 1